overall signal, the number of observed XBs was minimized to ~20. Autofluorescence and photobleaching were minimized by labeling the myosin lever arm with a relatively long-lived red-emitting dye containing a chromophore system encapsulated in a cyclic macromolecule. We show that the K104E mutation, when compared with Wild Type (WT) ventricles, had significant effect on both the kinetics of the interaction between actin and myosin and on the degree of order of the myosin lever arm. In particular, the K104E mutation increased the rate of XB binding to thin filaments and the rate of execution of the power stroke, while decreasing the rate of XB dissociation from actin. This implies that the mutated ventricle may be prone to decreased maximal tension and increased muscle relaxation time suggesting a potential for diastolic dysfunction in patients. Mutated XBs were significantly better ordered during steady-state contraction and during rigor but mutation had no effect on the degree of order in relaxed myofibrils.

2849-Pos Board B541

Measuring Work Loops in Intact Isolated Cardiac Myocytes by Controlling Pre- and Afterload using a New Generation Force Transducer

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A recent exciting development in cardiac research is the ability to do mechanical experiments on intact isolated cardiac myocytes. Here we mimic cardiac PV loops by imposing a 'pre-load' and 'after-load' on the myocyte. For these experiments we developed a new generation force transducer as currently available force transducers were not sufficiently sensitive or had insufficiently stable base line levels to achieve force control. A cantilever with a spring constant of 7 Nm is interrogated using an interferometer via an optical fiber. The resolution of the interferometer/probe system is 2nm, resonance frequency $>$ 2 kHz and force resolution $<$ 10 nN. As the probe is small enough to be fully submerged in water, variations in solution level have no effect on the force measurement, resulting in a stable baseline (drift $<$ 50 nN over a 10 minute period at 21C). Software was written to take in the signal from the force transducer, process it and return a signal to a linear motor that could stretch or shorten the myocyte in order to control force levels. Using this software we were able to achieve a two-sided force clamp (setting 'pre-load' and 'after-load') to measure work loops and re-create the Frank-Starling relation at the single cell level. Experiments show that in isolated cardiac mouse myocytes residual active force at the end of diastole limits the effective work the mycoyte can produce. Small concentrations of BDM, thought to inhibit strong crossbridge formation by stabilizing weak cross bridges, allow the myocyte to relax at end-diastole, shifting the pre-load-sarcomere length relation upwards. The resulting increase in length dependent activation outweighs the effects of crossbridge inhibition, leading to strongly increased mechanical work per contractile cycle.

2850-Pos Board B542

Temperature and Transmural Region Influence Functional Measurements in Unloaded Left Ventricular Cardiomyocytes

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Intact cardiomyocytes are increasingly being used to investigate the molecular mechanisms of contraction and to screen new therapeutic compounds. The function of the cardiomyocytes is often measured from the calcium transients and sarcomere length profiles. We studied the role of experimental temperature and transmural region on indices of function in freshly isolated, unloaded cardiomyocytes. Intact cardiomyocytes were isolated from the sub endocardium, mid myocardium and sub epicardium of 3 month old Sprague-Dawley rats. Myocytes from each region were studied at 25, 31, and 37°C. Cytosolic calcium transients were measured using Fura-2 fluorescence while sarcomere length shortening and relengthening profiles were measured using high speed video capture. For both the calcium transients and sarcomere length profiles, the time to peak and the time to half relaxation decreased significantly with increasing temperature. Increasing temperature also raised the minimum and maximum calcium levels of all cells. Of note, there was a reduced normalized standard deviation (standard deviation divided by the mean) at higher temperatures for calcium fluorescence amplitudes, time to peak calcium, and rates of sarcomeric shortening and relengthening. The amplitudes and minimum of the calcium transients were significantly dependent on transmural region, and several sarcomere length parameters exhibited statistical interactions between temperature and transmural region. Together, these results show that biological variability can be reduced by performing experiments at 37°C rather than at room temperature, and by isolating cells from a specific transmural region. Adopting these procedures will improve the statistical power of subsequent analyses and increase the efficiency of future experiments.

2851-Pos Board B543

A Novel Method for Isolating and Culturing Human Cardiomyocytes from Cryopreserved Tissues

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Current animal models fail to accurately mimic human heart biology. We have developed a novel method to isolate viable human cardiomyocytes (HCMs) from cryopreserved heart transplant donors. The Sydney Heart Bank is an effective and unique resource for isolating HCMs from donors as young as 2 months to 65 years. Preliminary data were collected from 2 donors aged 4 and 59 years. Left ventricle (LV) samples were frozen (−196°C) after being excised by transplant surgeons. LV tissues stored for 48 and 30 months, respectively, were then warmed gradually over 24 hours to 37°C. 300 μm thick cryosections were digested with collagenase type B and D [Mollova et al.. 2013 PNAS. 110:1446], gently agitated and then plated. Cell viability of isolated HCMs was evaluated using CellTracker CMFDA and calcein-AM. Our findings showed that isolated HCMs are metabolically active and viable. HCMs that were metabolically challenged using FCCP (trifluorocarbonylcyanide phenylhydrazone) increased their oxygen consumption. Immunohistochemical analyses using antibodies against cardiac markers showed that isolated HCMs express sarcomeric alpha-actinin, connexin43 and cardiac troponin T. Addition of BDM (2,3-butanedione monoxime, an inhibitor of myosin ATPase activity) allowed a much higher yield of viable HCMs (20-25% compared to 5% in the absence of BDM). However, the use of BDM in these culture seems to inhibit contractility of freshly isolated HCMs. Current studies are investigating optimal culture conditions to control contractility and viability of HCMs that will be used to replace current animal models. Taken together, our studies show that viable HCMs can be isolated and cultured from LV samples and may be used to investigate cardiomyocyte biology and pathology.

2852-Pos Board B544

Physiological Contractility of Cardiomyocytes in the Wall of Mouse and Rat Azygos Vein

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We recently demonstrated the abundant presence of cardiomyocytes in the wall of thoracic veins of adult mouse and rat (Kracklauer, Feng, Jiang, Lin, Lin, Jin, FEBS J. 280:880-91, 2013). The highly differentiated morphology and myofilament protein expression of the venous cardiomyocytes suggested contractile functions. Here we further investigated the contractility of mouse and rat azygos venous rings in comparison with that of atrial strips and ventricular papillary muscle. X-gal staining of transgenic mouse vessels expressing lacZ under cloned cardiac troponin T promoter demonstrated that the venous cardiomyocytes are discontinuous from atrial myocardium and longitudinally aligned in the wall of thoracic veins perpendicular to the vessel axis. Histology study showed striation patterns in the venous cardiomyocytes, which indicate encirclement orientation of myofibrils in the vessel wall. Mechanical studies found that mouse and rat azygos veins produce strong cardiac type twitch contractions when stimulated by electrical pacing in contrast to the weak and slow smooth muscle contractions induced using 90 mM KCl. The twitch contraction and relaxation of rodent azygos veins further exhibited cardiac type beta-adrenergic responses. Quantitative characterization showed that the contractions of venous cardiomyocytes are slightly slower than that of atrium muscle but significantly faster than that of ventricular papillary muscle. These novel findings indicate that the cardiomyocytes in rodent thoracic veins possess fully differentiated cardiac muscle phenotype despite their anatomical and functional separation from the heart.